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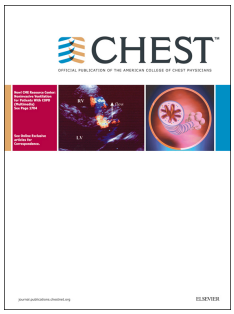

The potential role of aspiration in the asthmatic airway

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The potential role of aspiration in the asthmatic airway.

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Abstract

Background: Many asthmatics remain sub-optimally controlled despite current treatments. Reasons include comorbidities that could aggravate asthma, including gastro-esophageal reflux (GER). We aimed to investigate whether aspiration occurs in asthmatic patients and if so does it correlate with asthma control.

Methods: Patients had ACQ-7, FeNO, and spirometry performed to characterize their level of asthma control. Barium swallow with provocation was performed to assess for predisposition to aspiration. Patients underwent bronchoscopic investigation, with bronchoalveolar lavage (BAL) pepsin measured as a marker of aspiration.

Results: Seventy-eight patients stratified by disease severity (GINA) into mild (35.8%), moderate (21.7%) and severe (42.3%) were studied. Pepsin was detectable in BAL in 46/78 (58.9%). There were no differences between pepsin levels in patients with different disease severity. Furthermore, no significant associations were seen between pepsin level and measures of asthma control, FEV_1, ACQ or

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exacerbation frequency. Similarly no associations were found with adjustments for smoking history, BMI, proton pump inhibitor use, eosinophil count or IgE. When stratified into eosinophilic or neutrophilic asthmatic populations based on BAL there was no relationship to detected pepsin concentrations. A positive barium swallow (seen in 33/60 patients) did not correlate with BAL pepsin level and we found no significant association between barium swallow result and ACQ, GINA, exacerbation frequency or FEV_1 using either univariate or multivariate analyses.

Conclusion: Our study suggests that the importance of aspiration on current asthma symptom control and exacerbation rate may be over-stated. However, our study did not address the role of aspiration and future risk of exacerbation.

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INTRODUCTION

Approximately 10% of patients with asthma have persisting symptoms and exacerbations despite combination therapy with long-acting β agonists and inhaled corticosteroids[1]. This inadequate therapeutic response may be due to medication-refractory asthma or to difficult-to-manage asthma arising from confounding issues such as poor inhaler technique, poor adherence or coexisting comorbid disease[1-3]. Adherence to medication may be compounded by the fact that some patients may demonstrate a reasonable inhaler technique when directly observed, but may be careless in their inhaler use on a day-to-day basis[4-6]. Comorbidities and complicating conditions such as rhinosinusitis, gastroesophageal reflux (GER) and obesity also play significant roles in uncontrolled asthma[1]. □

Several risk factors have been identified with a more severe asthma phenotype. These include female sex, obesity, and tobacco exposure, some of which are also risk factors for GER[7]. Previous studies suggest that GER, although often asymptomatic, is common in the asthmatic population[8], but it is unclear whether any association between GER and asthma severity is the result of confounding or due to a direct independent effect. Trials examining the potential therapeutic use of proton pump inhibitors (PPI's) in asthma have had mixed results[9-11]. Therefore, current evidence does not support their routine use to improve clinical outcomes in asthma. However, while GER may predispose to aspiration it is not synonymous with it. In addition, treatment with PPI's reduces gastric acidity and thus acidity of reflux, but does not prevent non-acid reflux, or aspiration into the airways of at risk patients[12]. If such non-acid reflux is mechanistically important then PPI's would be expected to be of limited efficacy.

Pepsin is produced in the stomach in response to substances such as CCK, gastrin, VIP, secretin and insulin. Levels of pepsin, used as a biomarker of gastric aspiration, are elevated in BAL of lung transplant recipients, and are associated with acute and chronic rejection[14]. Fundoplication, which reduces or prevents aspiration has been successfully employed post-lung transplant in uncontrolled studies. Successful

fundoplication has also been reported in asthmatic patients, albeit in case reports and series[15].

The aim of this study was to determine the prevalence of aspiration (as defined by detectable pepsin in BAL) in a cohort of patients with mild, moderate and severe asthma. The association of BAL differential, smoking status, steroid use (oral as well as inhaled), PPI use, BMI as well as the presence of atopy, with pepsin level was also examined.

METHODS

Study approval was granted by the University College Cork's clinical research ethics committee: (ECM 4 (m) 01/10/13). After informed consent was received, 78 asthmatics were prospectively recruited from an asthma clinic in Cork University Hospital. On the morning of bronchoscopy, spirometry and a validated asthma control questionnaire (ACQ-7) were completed by enrolled subjects[16]. A history of exacerbations over the preceding 12-month period was ascertained and validated against both inpatient and community pharmacy prescriptions. Patients had blood drawn for peripheral eosinophil count, IgE and RAST testing for grass pollen, animal danders, house dust mite, and aspergillus. Finally testing of FeNO was performed using the NIOX MINO® device (Aerocrine AB, Sweden)[17].

Bronchoscopy and BAL:

Bronchoscopy was performed under conscious sedation with patients in a semi reclined position.

BAL was obtained from the right middle lobe or lingula as a standardized 3 x 60 ml procedure. BAL pH was measured using non-bleeding pH indicator strips immediately post-bronchoscopy (Merck, Germany). The BAL sample was divided, and clinical microbiology was assessed in a standardized fashion. Differential cell counts were made on Kwik Diff™ stain (Thermo Scientific, USA) cyto-centrifuge preparations. Cell-free BAL supernatants were prepared by centrifugation (10 min, 500g, 21°C); aliquots were subsequently stored at -80°C before ELISA testing.

Pepsin ELISA

A locally developed indirect ELISA was used to measure pepsin[13]. Briefly, 100 µL of pepsin standard (pepsin from porcine gastric mucosa, Sigma) and undiluted BAL supernatants were allowed to adsorb onto a 96-well plate (Nunc MaxiSorp) in triplicate. Non-specific binding sites were blocked with 1% ELISA grade bovine serum albumin (Merck Millipore) in PBS (pH 7.4). The primary antibody was specific to porcine pepsin (Biodesign International Cat no W59117G). Secondary antibody was horse radish peroxidase-conjugated rabbit, anti-goat (Sigma). Antibodies were diluted in Tris-buffered saline (20 mM Triza base, 150 mM NaCl, pH 7.4) containing 0.1% BSA and 0.05% Tween 20. The substrate used was 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and the reaction was stopped with an equal volume of 1% SDS. Negative controls were performed with the omission of primary antibody.

Barium Swallow

A standard double-contrast barium swallow study was carried out with patients in both upright and supine positions. Where spontaneous GER was not demonstrated, provocation manoeuvres were employed, starting with a cough, followed by Valsalva manoeuvres, rolling the patient and the water siphon test. Secondary signs of reflux were recorded, including esophageal reflux, cricopharyngeal bars and hiatal herniae but only patients in whom reflux was demonstrated during the procedure were characterised as positive for GER[18].

Statistics:

The distribution, central tendency and spread of each variable were examined using standard tabular and graphical methods. In primary analysis the association of aspiration with markers of disease severity was examined in univariate analysis using Fishers exact test and in multivariate analysis using logistic regression. Analysis was conducted using GraphPad Prism 6 (La Jolla California USA).

RESULTS:

Patient characteristics are described in Table 1. Seventy-eight patients were recruited (49 female). There were 8 current (14.8 pack years), 19 ex-smokers (15 pack years) and 51 non-smokers. There were no significant differences in smoking status across disease severity.

Thirty-two patients demonstrated positive barium studies. Twenty-six patients were on PPI's and/or H_2-antagonist therapy. Eight patients were on maintenance regimens of oral steroids at the time of testing (range 5-20mg daily). In total 63 patients underwent all investigations, with a further 15 patients undergoing all investigations except barium swallow.

Pepsin levels:

BAL pepsin was detectable in 46/78 (58.9%) patients. Peripheral eosinophilia (>$0.3x10^9$/ml) was present in 26/78 (33.3%), with an elevated IgE (>80UI/ml) detected in 42/78 (53.8%) of patients. BAL cell differential demonstrated eosinophilia (>1%) in 28/78 (35.9%), and neutrophilia (>3%) in 27/78 (34.6%) (see Supplemental Figure 1)[19]. A significant correlation was seen between peripheral and BAL eosinophils, r= 0.39 ($p < 0.0005$).

There was no association between detectable pepsin and disease severity, asthma control, exacerbation frequency or FEV_1 (Fig. A). Similar results were found when the model was further adjusted for smoking history, BMI, PPI use (Fig B), eosinophil and neutrophil counts and serum IgE. BAL pH also had no significant association with disease severity (Figure C).

There was no significant difference between barium study result and BAL pepsin; mean (sd) pepsin for positive study 3.4 (1.3) vs. 3.8 (1.4) ng/ml for negative study, p= non-significance (Fig D). When the cohort was divided into positive/negative barium swallow, there was no association between positive barium swallow and ACQ, asthma severity, exacerbation frequency or FEV_1 on either univariate or multivariate analyses.

In a simultaneous multivariable linear regression model, ACQ scores were

significantly related to oral corticosteroid use (β=1.2, p=0.006), inhaled corticosteroid use (β =0.83, p<0.001), but not detectable BAL pepsin (β =0.004, p=0.99).

DISCUSSION:

Research to date, pertaining to the possible utility of BAL pepsin as a marker of aspiration has been undertaken mainly in the areas of acute lung injury, end-stage lung disease, and post lung transplantation[13,14,23]. It has been shown that detection of pepsin and bile acids in BAL and exhaled breath condensate can be employed to aid identification of lung transplantation recipients with GER-induced aspiration. Elevated levels are associated with acute allograft rejection and/or bronchiolitis obliterans syndrome[13]. The use of pepsin as a surrogate for aspiration and therefore increased risk of rejection has led to its use as a means for justifying anti-reflux surgery in this niche population, with clinical outcomes suggesting a protective role in terms of non-allogenic injury[20].

It has long been postulated that there is a link between GER and asthma severity[7]. Despite this there has been a paucity of research specifically looking at airway pepsin as a biomarker of adverse clinical outcome and disease severity in the asthmatic lung. Our results demonstrated a median pepsin concentration of 3.58ng/ml (range 2.53-15.78ng/ml), in the 58.9% of patients demonstrating detectable pepsin, confirming that aspiration does occur in asthmatic patients. Despite its demonstrable presence, pepsin concentration showed no significant association with clinical markers of disease severity or asthma control.

Asthma and GER are known to share potential risk factors, such as smoking and obesity. BAL pepsin levels demonstrated no association with either BMI or smoking history. More importantly, there was no significant difference in pepsin levels when divided according to the presence or absence of GER by barium swallow.

The median pepsin concentration of 3.58ng/ml, when compared to findings in other studies (using similar BAL sampling techniques), was significantly greater than control groups (median 1.1ng/ml), and patients with chronic cough (median 0ng/ml). This was therefore consistent with the presence of micro-aspiration into the lungs of some asthmatics[13]. The levels are below those seen post lung transplantation (median

8.3ng/ml), which is not surprising as lung allograft recipients are regarded as being especially vulnerable to aspiration because of gastroparesis, impaired cough[21], and dysfunctional mucociliary clearance[22].

Pepsin levels however did not exhibit a significant correlation with asthma exacerbation frequency (p=0.66). When patients were subdivided into groups by exacerbation frequency (≤3, 4-6 and ≥7 exacerbations/year) there was no significant difference in pepsin concentration across the groups (3.27ng/ml, 2.53ng/ml and 3.51ng/ml respectively). There remains the possibility that low-grade exposure to aspiration over a prolonged period of time may still play a role in promoting airway inflammation and increase the risk of future exacerbation, but our study does not suggest that this is associated with poorer asthma control.

Pepsin levels were not significantly different in patients treated with maintenance acid suppression therapy (median, 2.85ng/ml) compared with patients who were untreated (median, 2.79ng/ml p =ns). This provides evidence of gastric aspiration even in patients who were treated with maintenance PPI's. Similar results have been previously reported in the post-transplant population[14]. This may partially explain why trials examining the potential use of PPI's in asthma have afforded mixed results[9-11,23].

In this study we utilized pepsin and barium swallow (with provocation), rather than pH manometry/24hr impedance to assess reflux. Barium swallow (esophagography) maintains a role in the modern assessment of GERD, especially in patients with symptoms severe enough to warrant the consideration of surgical intervention[24-28]. Despite it's many limitations, a recent consensus statement from the Society of Abdominal Radiology has recommended that due to its inexpensive and non-invasive nature, in conjunction with its wide availability, that Barium swallow should be considered as a first-line test for the investigation of GERD[29]. While the sensitivity of the standard barium swallow for the detection of reflux is often quoted as low as 35-50%[30], with the addition of provocative manoeuvres the sensitivity of barium swallow approaches that of pH testing[31,32]. As we were more interested in the role of aspiration, (be it acidic or non-acidic refluxate) we felt that barium swallow (at the time of initial study planning and approval) was an appropriate means to assess for

reflux with the presence of BAL pepsin ultimately defining the presence of aspiration. Our results demonstrated however, no significant relationship between barium swallow and either pepsin concentration or exacerbation frequency, thereby suggesting that Barium swallow did not accurately reflect the presence of aspiration in our cohort. Although we did not assess for noctural reflux symptoms, across the entire asthma disease severity there was no observable relationship to the presence of aspiration or the treatment effect of PPI. Furthermore, our study would undoubtedly have been strengthened by performing pH manometry/24hr impedance in our patients and exploring whether or not this test reflects BAL pepsin levels.

Further studies have looked at the effect of PPIs on acidic bile salts as opposed to pepsin. Hou *et al* demonstrated in an epithelial cell model that omeprazole inhibits IL-8 secretion stimulated by exposure to acid and bile salts[33]. They demonstrated that acidic bile salts activate the IL-8 promoter through NF-KB and AP-1 DNA binding sites, and that omeprazole inhibits IL-8 production by blocking nuclear translocation of p65, as well as decreased binding of p65, c-jun and c-fos to the IL-8 promoter. These effects occurred independently of the effects on gastric acid secretion, demonstrating a novel mechanism that might contribute to the beneficial effects of PPIs in the treatment of reflux related pulmonary inflammation. The caveat to this finding comes from research published by Pauwels *et al*, in which they examined the effect of gastric aspirates from CF patients being treated with a PPI and found that such aspirates demonstrated an enhanced inflammatory effect (higher IL-8 production) on CF bronchial epithelial cells[34]. We have recently shown that microbiological growth from gastric juice occurred when gastric juice pH was >4 and that gastric and airway microbiomes of people with CF who reflux are similar, indicating a possible mechanism to explain such observations[35,36]. As chronic PPI treatment in CF may result in a paradoxically proinflammatory effect in airways, alternative anti-reflux therapies may need to be considered.

Our study has several possible limitations. Having only 17% of our patients designated GINA 5, limits the strengths of our conclusions. Furthermore, we did not assess another commonly cited asthma outcome measure; bronchial hyper-reactivity. This would undoubtedly have been of interest, and would have enhanced our findings.

There also exists a plausible alternative mechanism for reflux-mediated exacerbation of asthma in the form of vagally mediated, reflexive bronchospasm. Acidic-stimulation of esophageal mucosal receptors giving rise to vagally mediated reflex bronchospasm. Mansfield and colleagues noted that the total respiratory resistance increased by 10% in asthmatics with a positive Bernstein test (acid perfusion test) after esophageal acid stimulation[37]. Wright and colleagues noted significant decreased airflow and arterial oxygen saturation before and after esophageal acid perfusion[38]. Atropine pretreatment abolished these findings, providing evidence for an acid-induced vagally mediated esophagobronchial reflex. Therefore, although our results show definitive aspiration (as denoted by the presence of pepsin in the BAL of our cohort); the possibility remains for worsening of asthma symptoms without aspiration into the lungs, but simply into the esophagus, potentially via this alternative mechanism.

We have demonstrated that pepsin, and therefore aspiration occurs in the asthmatic lung. However, there does not seem to be a clear link between its presence and disease severity. It may be that in asthmatics, pepsin in itself is not an accurate biomarker of the inflammatory airway process that results from exposure to chronic aspiration. Alternatively, it may be that chronic exposure over time to low levels of aspirate is sufficient to drive an inflammatory response causing subsequent differences in clinical severity. Further studies at cellular level are needed to examine the underlying pathways involved and ascertain whether the presence of pepsin is indeed a marker of a pro-inflammatory state. However, based on the lack of clearly demonstrable difference between pepsin levels in patients with differing asthma severity, it may ultimately be that the impact of aspiration on disease severity at a clinical level has been over stated.

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DM had access to all data in the study and takes responsibility it's integrity and the accuracy of data analysis. EH, CW, SP, AS, JP, SL, PO'B, JE, BP, MM and JMacS contributed substantially to study design, data analysis and interpretation, and writing of the manuscript. We thank Professor Elizabeth Juniper for permission to use the ACQ-7.

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TABLE 1. PATIENT CHARACTERISTICS

(Mean ± Standard deviation)

	N=	ACQ-7 score	FEV_1 (%predicted)	Blood Eosinophil count(10^9/ml)	BMI (kg/m^2)	pH	Pepsin (ng/ml)	FeNO (ppb)	Exacerbations/ year
GINA 1	11	1.2 (0.7)	80.9 (10.8)	0.22 (0.22)	26.16 (5.33)	6.16 (0.30)	5.88 (4.14)	37.8 (32.1)	2.18 (1.72)
GINA 2	17	1.1 (0.6)	90.0 (13.6)	0.29 (0.23)	25.76 (3.91)	6.22 (0.20)	4.68 (1.55)	30.2 (23.5)	3.00 (3.92)
GINA 3	17	1.9 (1.0)	86.7 (20.6)	0.33 (0.39)	27.54 (6.87)	6.16 (0.34)	4.45 (1.61)	24.1 (10.6)	3.63 (2.36)
GINA 4	20	2.7 (0.7)	75.5 (17.1)	0.20 (0.17)	29.61 (6.45)	6.23 (0.37)	4.43 (1.39)	20.2 (14.3)	4.75 (3.65)
GINA 5	13	4.2 (1.1)	47.7 (20.2)	0.35 (0.32)	31.13 (6.16)	6.33 (0.44)	4.02 (1.52)	32.1 (43.4)	8.23 (4.60)

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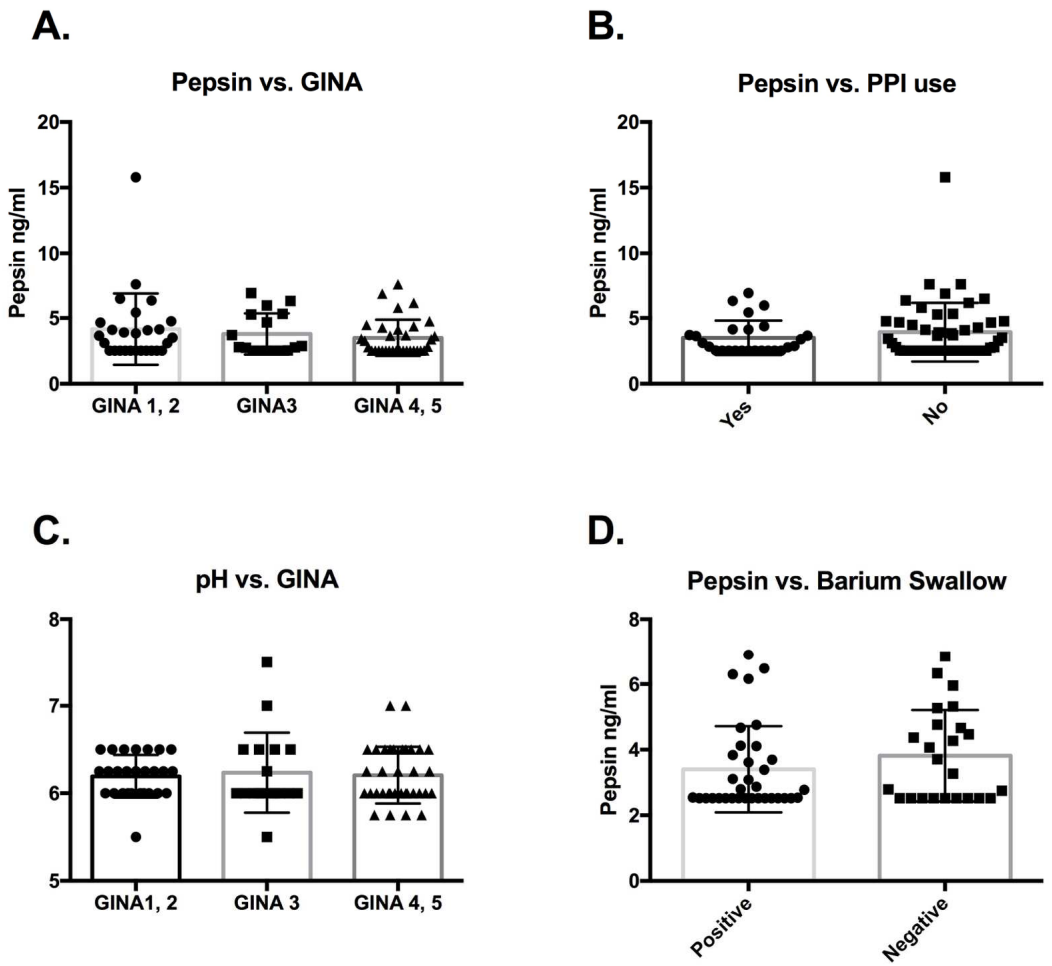

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Abbreviation List

BAL: bronchoalveolar lavage

BMI: body mass index

FeNO: fractional exhaled nitric oxide

FEV_1: forced expiratory volume in one second

GER: gastro-esophageal reflux

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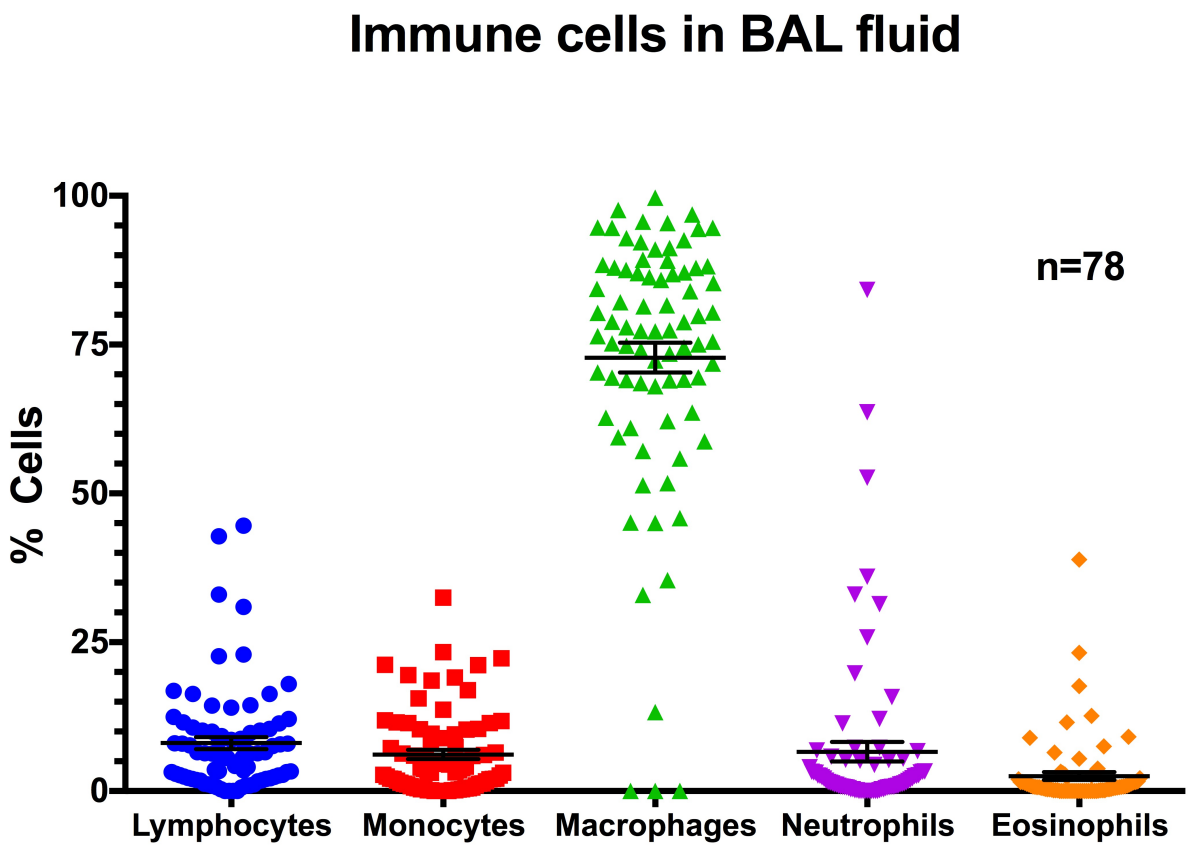

e-Figure 1. BAL Immune cell profile of asthmatic patient cohort. n= 78.

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